Lasers in Surgery and Medicine 20:443–450 (1997)

In Vitro Effects and Localisation of the Photosensitizers m-THPC and m-THPC MD on Carcinoma Cells of the Human Breast (MCF-7) and Chinese Hamster Fibroblasts (V-79)

R. Hornung, MD,[1] B. Jentsch,[1] N.E.A. Crompton, PhD,[2] U. Haller, MD,[1] and H. Walt, PhD[1]*

[1]Research Division of Gynecology, Department of Gynecology and Obstetrics, University Hospital, CH-8091 Zurich
[2]Institute of Medical Radiobiology of the University of Zurich and the Paul Scherrer Institute, Villigen, Switzerland

Background and Objective: **Photodynamic therapy (PDT) is the combination of a photosensitizer with laser light to induce preferential destruction of malignant cells. In this study two new photosensitizers—5,10,15,20-meta-tetra(hydroxyphenyl) chlorin (m-THPC) and m-THPC MethoxyPEG2000 derivative (m-THPC MD)—were tested, both for their dark toxicity, i.e., cytotoxicity in the absence of light, and for their light-induced cytotoxicity in mammalian cell cultures.**
Study Design/Material and Methods: **Cell lines used were MCF-7 (human breast carcinoma) and V-79 (Chinese hamster lung fibroblast). After cultivation under standard conditions, cells were administered the photosensitizers and 24 hr later exposed to various energy levels of laser light at a wavelength of 652 nm. Cell survival was monitored using a clonogenic assay and was expressed as the surviving fraction of the untreated control.**
Results: **Up to an m-THPC concentration of 1 μg/ml, no dark toxicity was observed; at higher concentrations a rapid fall in survival occurred. m-THPC MD showed no dark toxicity up to 100 μg/ml. In vitro m-THPC was ~10 times more cytotoxic than m-THPC MD. The MCF-7 and V-79 cell lines displayed similar responses to PDT.**
Conclusions: **Both m-THPC and m-THPC MD are very efficient photosensitizers in vitro. Up to the therapeutic dose, neither exhibited dark toxicity. There is clinical relevance of the photosensitizers by a large therapeutic index. Lasers Surg. Med. 20:443–450, 1997.** **© 1997 Wiley-Liss, Inc.**

Key words: human and animal cell cultures; dark toxicity; photodynamic therapy

INTRODUCTION

PDT is a novel alternative to surgery, radiotherapy, and chemotherapy for treatment of malignant tumours. Cell death is induced by a photosensitizer following exposure to light matching the sensitizer absorption. At the beginning of the century, it was already known that tumour tissues enrich autogenous pigments, especially porphyrins, which efficiently absorb sunlight. In some cancer cases, pigment enrichment resulted in relief of the disease [1]. With this observation, the foundation stone of PDT research was laid. Contemporary PDT research involves study of both its

*Correspondence to: Heinrich Walt, Research Division of Gynecology, Department of OB/GYN, University Hospital, CH-8091 Zürich, Switzerland.

Accepted for publication 1 August 1996.

constituent modalities: optimisation of the properties of laser light for cancer treatment, and a worldwide effort to find more efficient photosensitizers [2]. PDT photosensitizers should display at least the following two properties: a selective affinity for malignant tumours [3,4] and minimal cytotoxicity in the absence of light. Many photosensitizers already have been developed and tested, such as the hematoporphyrin derivative (HpD, Photosan 1–3, Photofrin II), the phthalocyanins, various benzoporphyrin derivatives (BPD), and others. These all display either low efficiency or unacceptable side effects. All photosensitizers are dyes, most of them are derivatives of the porphyrins. Bonnett et al. [5,6] defined the following criteria that should be fulfilled by "second-generation" photosensitizers: they should lack toxicity in the absence of activating light, they should have a uniform stable composition preferably being a single substance, they should display specific affinity for tumour tissue (a feature that appears to be related to their amphiphilic properties), and they should absorb red light because of the enhanced tissue-penetrability of these wavelengths. With modifications to the structure of porphyrin and its ligands, photosensitizers of the second generation were developed. A shift in the maximal light absorption to red light was achieved with reduction of porphyrin to chlorin. The properties of the dyes 5,10,15,20-meta-tetra(hydroxyphenyl)chlorin (m-THPC) and m-THPC MethoxyPEG2000 derivative (m-THPC MD) seem to be particularly suitable. Studies have already demonstrated greater cytotoxicity of these two agents, and they also display greater selectivity for malignant tumours [7].

The aims of the present study were to characterise the photosensitizers m-THPC and m-THPC MD with respect to their dark toxicity and their light-induced cytotoxicity on both the human breast carcinoma cell line, MCF-7, and a lung fibroblast cell line of the Chinese hamster, V-79. Moreover we were interested in the localisation of the photosensitizers in the cells chosen for this study.

MATERIALS AND METHODS

Two different mammalian cell lines were used for these studies: The estrogen-dependent MCF-7 cell line was derived from a human breast cancer and had a doubling time of 1.3 days. The V-79 cell line originated from Chinese hamster lung fibroblasts and had a doubling time of 11 hr. Cells were cultured in Opti-MEM medium (Gibco, Basel, Switzerland) supplemented with 10% fetal calf serum, 25IE/ml Penicillin, and 25 mg/ml Streptomycin. The cells were incubated in a gas incubator with 37°C, 5% CO_2, and 100% humidity. The photosensitizer m-THPC (5,10,15,20-meta-tetra(hydroxyphenyl)chlorin; Foscan®, Scotia Pharmaceuticals, Guildford, UK) was prepared in an ethanol:polyethylene-glycol:water, 20:30:50, solution [8]. Its watersoluble counterpart m-THPC MD (m-THPC MethoxyPEG2000 derivative; Scotia Pharmaceuticals) was prepared in a 1:1 water solution. The optimal stimulation wavelength of both photosensitizers m-THPC and m-THPC MD for PDT is 652 nm. Two working concentrations of the photosensitizers were chosen: 0.1 μg/ml and 1 μg/ml. Because m-THPC MD has a higher molecular weight than m-THPC, the quantity of MD in this report is given in terms of the active m-THPC contained in the m-THPC MD. This allows equimolar amounts of the compound to be compared. A series of experiments were performed with the ethanol:polyethylene-glycol:water, 20:30:50, solution alone in order to assess its potential toxicity in the dark.

Survival was determined based on the colony formation assay of Puck and Marcus [9]. MCF-7 cells required 10 days of posttreatment growth to produce reasonably sized colonies. V-79 cells required only 5 days. Between 100 and 10^6 cells were plated into Petri dishes with a diameter of 5 cm depending on expected survival and were incubated for 24 hr. The photosensitizers were then added, and 24 hr later the cells were exposed to red light. From the studies of Ris et al. [10], it is known that the effect of PDT depends on the drug-light interval. PDT is most effective after a 12-hr drug-light interval and diminished gradually thereafter. Based on these findings, we chose a drug-light interval of 20 hr.

The irradiation was carried out using either an Argon ion laser pumped-dye laser (Coherent Innova 310 and CR 599, GMP, Re, Switzerland), or a diode laser (Applied Optronics, South Plainfield, USA). The laser light was conducted through a glass fibre to the site of cell exposure. To diminish the influences of scattered light, the room was dimmed during irradiation. A final output of 25 mW and energy-doses of 0.35, 1.05, 2.11, 4.23, and 8.45 J/cm^2 were administered. After suitable periods of posttreatment growth, to permit colony formation, the cells were fixed and then stained with May-Gruenwald-Giemsa (azur-eosine-methylene blue). The clonogenic assay has

been used by various authors to examine PDT on MCF-7 cells, e.g. [11], and was adopted here for comparative purposes. After about six cycles of mitosis, surviving cells grew to clones of 50 cells or more. This was the minimum size used when scoring a clone of cells as a colony. The number of colonies was expressed as a fraction of the untreated control. Each trial was repeated at least three times in order to determine standard errors. Statistical significance of important values was calculated with the Mann-Whitney-U test. P values <0.05 were considered as statistically significant.

For the localisation of the dyes, the cells were cultivated on glass slides for 1 day. Then 1 μg/ml of either m-THPC or m-THPC MD was applied and incubated for 22 hr. For the detection of fluorescence, we used a confocal laser scanning microscope (Leica, TCS 4D, Glattbrugg, Switzerland), which allows us to image thick fluorescent-labelled biological material by using optical cell slicing [22,23]. Typical views were chosen and printed.

RESULTS

No toxicity from either photosensitizer was observed up to a concentration of 1 μg/ml when MCF-7 cells incubated with either m-THPC or m-THPC MD, but not exposed to red light (Fig. 1). Above 1 μg/ml, survival in m-THPC decreased rapidly so that at 10 μg/ml, the fraction of survival was 9.7×10^{-4} ($\pm 4.8 \times 10^{-4}$) of controls ($P < 0.05$). A decrease in survival following m-THPC MD was not observed until concentrations higher than 100 μg/ml were given. The ethanol:polyethylene-glycol:water, 20:30:50 solution of m-THPC showed a survival fraction of 0.811 (±0.015) at a drug concentration of 100 μg/ml. Survival of the V-79 cells showed a similar response to the two photosensitizers (Fig. 1).

Cell survival was examined of MCF-7 and V-79 cells exposed to 652 nm red-light but in the absence of photosensitizers (Fig. 2). Surviving fractions higher than 1 were observed up to energy-doses of 8.45 J/cm^2. The curves for both cell lines were essentially identical, passing through a maximum at an energy-dose of 2.11 J/cm^2 of 1.4 (±0.3) for MCF-7 cells and 1.1 (±0.1) for V-79 cells.

The survival of MCF-7 cells after PDT treatment was tested with both photosensitizers at concentrations of 0.1 and 1 μg/ml (Fig. 3). Survival after 0.1 μg/ml m-THPC and 0.35 J/cm^2 red light fell to 0.4 (±0.2) and with 8.45 J/cm^2 to 5.9 $\times 10^{-4}$ ($\pm 2.6 \times 10^{-4}$). The corresponding values for 0.1 μg/ml m-THPC MD were 0.7 (±0.1) and 0.5 (±0.1; $P < 0.05$), respectively. Even at low m-THPC concentrations, PDT displayed a clear

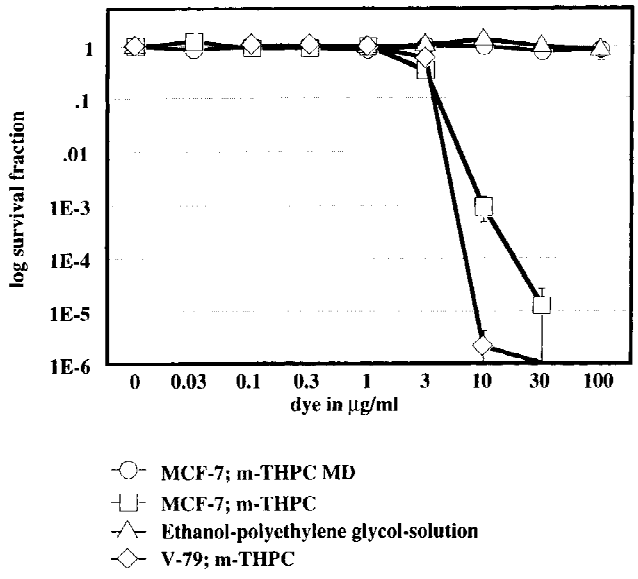

Fig. 1. Dark toxicity of m-THPC and m-THPC MD on MCF-7 and V-79 cells (bars indicate the standard error of mean). Up to an m-THPC concentration of 1 μg/ml, no dark toxicity was observed, however, at higher concentrations a rapid fall in survival occurred ($P < 0.05$). m-THPC MD showed no dark toxicity up to 100 μg/ml. Similarly, the ethanol:polyethylene-glycol:water (PEG) solution of m-THPC did not show relevant cytotoxicity. The effect of m-THPC MD on V-79 cells is almost identical with those on MCF-7 cells with m-THPC MD and the PEG-solution. Because these curves overlap, these data were not graphically presented.

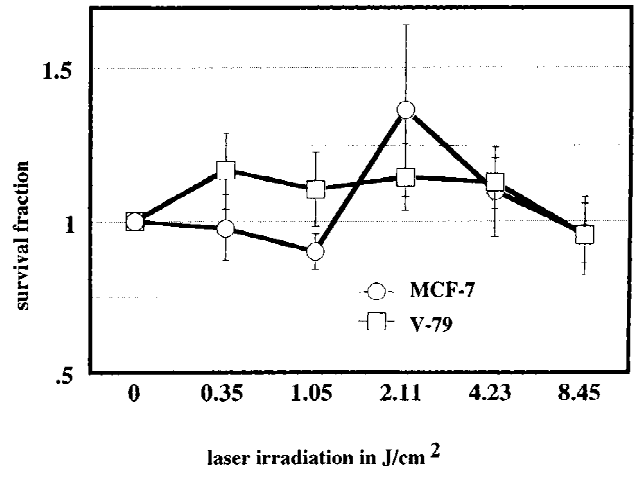

Fig. 2. Effects of laser irradiation on MCF-7 and V-79 cells (bars indicate the standard error of mean). Laser irradiation up to 8.45 J/cm^2 did not induce cell death, but tended to promote cell proliferation.

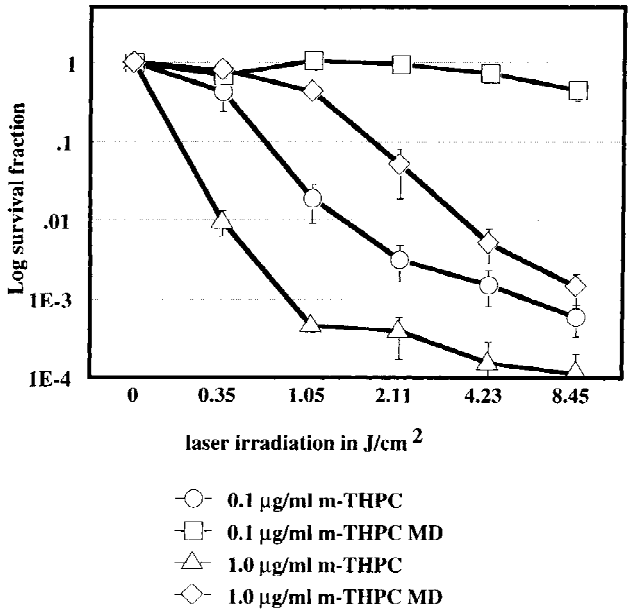

Fig. 3. Survival fraction of MCF-7 cells after PDT with m-THPC or m-THPC MD (bars indicate the standard error of mean). In vitro m-THPC was ~10 times more cytotoxic than m-THPC MD (at 8.45 J/cm^2 $P < 0.05$ for the lower and $P < 0.01$ for the higher concentration). Both photosensitizers showed decreasing survival fraction with both increasing concentration or light intensity.

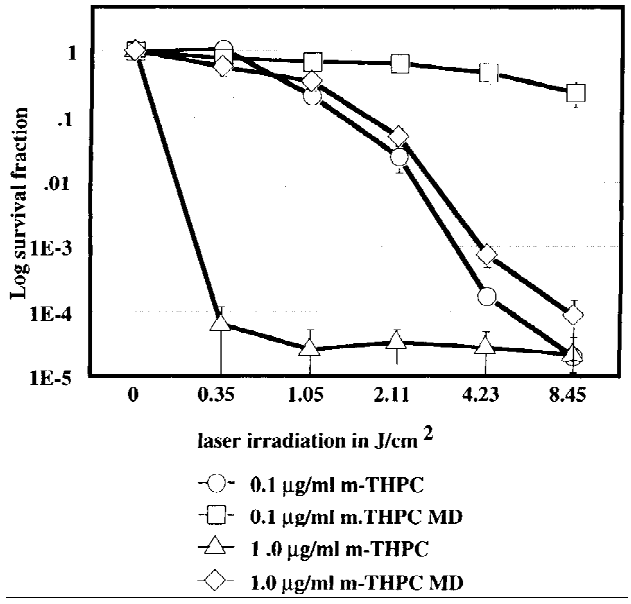

Fig. 4. Survival fraction of V-79 cells after PDT with m-THPC or m-THPC MD (bars indicate the standard error of mean; at 8.45 J/cm^2 $P < 0.01$ for 0.1 µg/ml photosensitizer). Both m-THPC and m-THPC MD were very efficient on V-79 cells. At high m-THPC MD concentrations, an increasing cytotoxicity with higher red light intensities was observed. m-THPC had already reached high levels of cell kill at the lower light intensities, and little extra kill with increasing fluence-doses was observed.

dose-response relationship with increasing laser intensity, whereas PDT with low m-THPC MD concentrations displayed little cytotoxicity. With 1 µg/ml m-THPC and 0.35 J/cm^2 red light, survival fell to 9.6×10^{-3} ($\pm 3.4 \times 10^{-3}$) and with 8.45 J/cm^2 to 1.7×10^{-4} ($\pm 1.1 \times 10^{-4}$). The corresponding values for 1 µg/ml m-THPC MD were 0.8 (±0.2) and 1.4×10^{-3} ($\pm 6.7 \times 10^{-4}$; $P < 0.01$), respectively. At high m-THPC MD concentrations, an increasing cytotoxicity with higher red-light intensities was observed. m-THPC had already reached high levels of kill at the lower light intensities, and little extra kill with increasing energy-doses was observed.

With 0.1 µg/ml m-THPC and 0.35 J/cm^2 red light, survival of V-79 fell to 1.1 (±0.2) (Fig. 4). With 8.45 J/cm^2, survival fell to 2.0×10^{-5} ($\pm 8.6 \times 10^{-6}$). With the higher 1 µg/ml m-THPC concentration and 0.35 J/cm^2 red light, survival fell to 6.1×10^{-5} ($\pm 6.6 \times 10^{-5}$) and with 8.45 J/cm^2 red light, it fell to 2.2×10^{-5} ($\pm 1.9 \times 10^{-5}$). At the lower concentration of m-THPC, PDT effect increased with increasing laser intensity. With the higher m-THPC concentration, a higher cell kill was observed even with low light intensities. Increasing the laser intensity resulted in little extra killing. PDT with 0.1 µg/ml m-THPC MD and 0.35 J/cm^2 red light showed a survival fraction of 0.8 (±0.2) and with 8.45 J/cm^2 of 0.2 (±0.1; $P < 0.01$). With 1 µg/ml m-THPC MD, the corresponding values were 0.6 (±0.2) and 8.6×10^{-5} ($\pm 6.8 \times 10^{-5}$; n.s.), respectively. At the lower concentration of m-THPC MD, little cytotoxic effect on the V-79 cells was observed. The curve of 1 µg/ml m-THPC MD was very similar to the curve of 0.1 µg/ml m-THPC indicating that m-THPC was ~10 times more cytotoxic than m-THPC MD. When MCF-7 cells were incubated with m-THPC for 22 h, the photosensitizer was distributed all over the cytoplasm with the highest fluorescence intensity located around the nucleus (Fig. 5). The nucleus did not show more than its autofluorescence. V-79 cells showed a similar distribution of m-THPC (Fig. 6), but with a narrow cytoplasm, typical for fibroblasts. Whereas the dye was homogeneously distributed in the perinuclear area of the MCF-7 cells, in the V-79 cells, there were areas with considerably higher fluorescence than elsewhere in the cytoplasm.

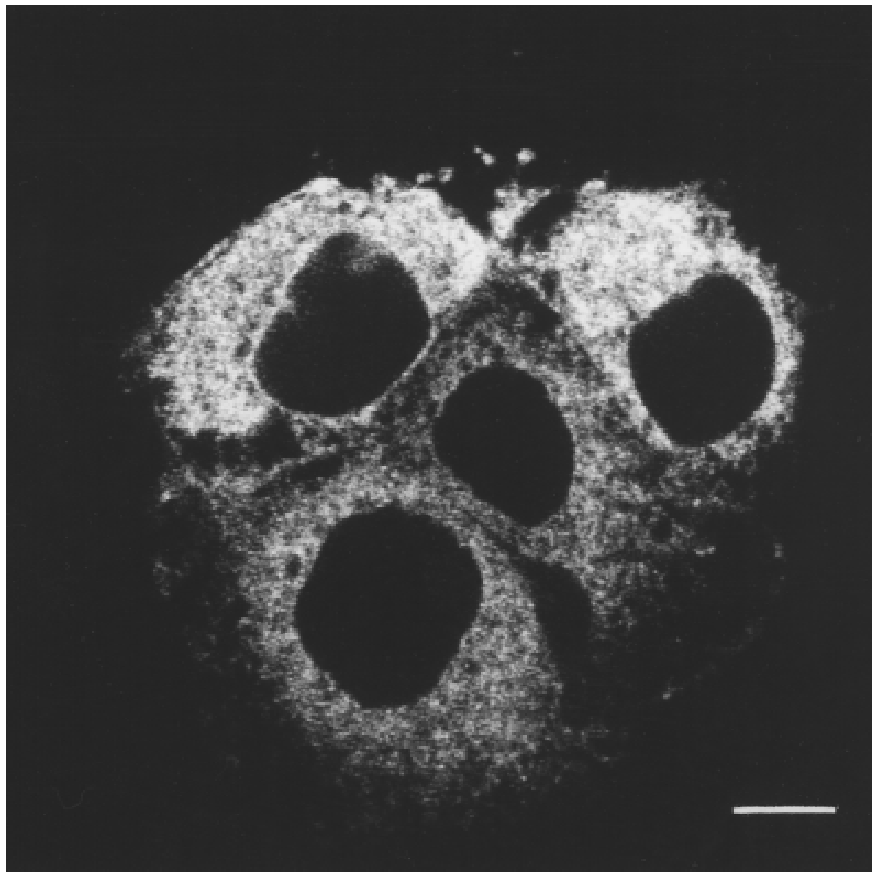

Fig. 5. Localisation of m-THPC induced fluorescence in MCF-7 cells. The dye is distributed all over the cytoplasm with the highest fluorescence intensity located around the nucleus. The nucleus displayed only autofluorescence (micrograph by confocal laser scanning microscopy, bar = 10 μm).

DISCUSSION

Cells enriched with PDT photosensitizers in their cytoplasms are killed after exposure to laser light, especially with laser light of defined wavelength. In this study the effects of the photosensitizers m-THPC and m-THPC MD on the cell lines MCF-7 and V-79 were investigated. Dark toxicity is one of the most important criteria for assessing the usefulness of a photosensitizer. The concentrations proposed by Ris et al. [12] for in vivo therapy (0.3 mg/kg body weight) are comparable to the concentrations used in this study for in vitro testing (1 μg/ml). Both sensitizers, m-THPC as well as m-THPC MD, showed no dark toxicity up to this concentration. Whereas m-THPC at elevated concentrations increased in its toxicity, even in the absence of light, this was not observed for m-THPC MD at concentrations 100 times higher. We could demonstrate that the difference of the cytotoxicity was not due to the ethanol:polyethylene glycol:water, 20:30:50 mix in the m-THPC solution, as shown in Figure 1. This study clearly demonstrates the superiority of m-THPC MD to m-THPC in respect to dark toxicity. Clinical relevance comes from therapeutic index of the photosensitizer. Therefore, the concentration range by which a drug starts to be effective and in parallel begins to display lethal side effects should be as large as possible. Based on these criteria, m-THPC MD is an optimal drug.

Several studies have shown that laser light at low intensities stimulates the proliferation of fibroblasts [13]. Survival of both MCF-7 and V-79 cells was increased after exposure to red light alone. In accordance with other authors [14], a clear dose-response relation of this growth stimulation was observed. With low intensity laser light, more clones survived than in the unirradiated control. Higher intensity laser light alone reversed this effect. Several mechanisms for the

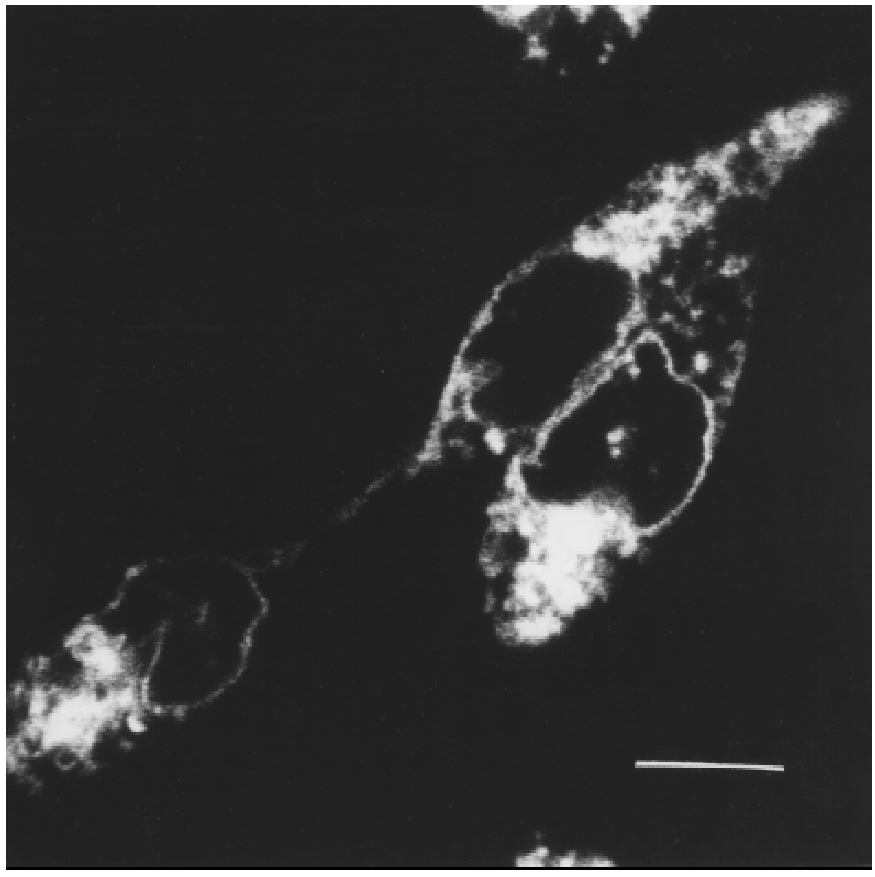

Fig. 6. V-79 cells with m-THPC induced fluorescence. V-79 cells show an inhomogeneous distribution of the photosensitizer within the cytoplasm. The nucleus is free from m-THPC induced fluorescence (micrograph by confocal laser scanning microscopy, bar = 10 μm).

stimulating effect of low-level red light exposure have been proposed, including an increased transmembrane electrochemical proton-gradient in mitochondria [15]. This proton-gradient increase would cause enhanced calcium release into the cytoplasm leading to a triggering of mitosis and enhanced cell proliferation. At higher doses, cell damage could result from an enhanced calcium influx, leading to ATP depletion and stimulated production of proteases and lipases, which affect the cytoskeleton and membranes, respectively. Recently, Yu et al. [13], demonstrated that basic fibroblast growth factors were stimulated by laser irradiation. Currently red-light induced stimulation of fibroblasts is being used to promote wound healing in various clinical situations.

Comparison of the effects of m-THPC vs. m-THPC MD on the breast cancer cell line MCF-7 demonstrated a relatively low cytotoxicity of 0.1 μg/ml m-THPC MD at the various red-light fluence-doses studied; 1 μg/ml m-THPC MD was slightly less cytotoxic than 0.1 μg/ml m-THPC. Under the conditions of the present study, m-THPC was 10 times more cytotoxic than m-THPC MD. This may result from different mechanisms of photosensitizer uptake by the cell with consecutively different cellular concentrations. Dobler-Girdziunaite et al. [16] investigated the photosensitizers zinc-phthalocyanine (ZnPc) and hematoporphyrin-derivative (HpD); 4 μg/ml ZnPc and 11.2 J/cm^2 red light resulted in a 10% survival; 4 μg/ml HpD and 8.4 J/cm^2 red-light resulted in a 70% survival. Compared with the present study, m-THPC is much more potent than both ZnPc and HpD. Singlet oxygen has been considered to be the cytotoxic principle involved in PDT [17]. Energy transfer from the activated photosensitizer to molecular oxygen results in production of singlet oxygen, which causes DNA strand breaks and membrane damage to mito-

chondria, lysosomes, the plasma membrane, and endoplasmic reticulum [14]. We could show that the photosensitizers were not located within the nucleus. They were distributed within the cytoplasm. Dobler-Girdziunaite et al. [18] have shown that there is a high fluorescence intensity in the mitochondria. If DNA strand breaks are the reason for the cell death after PDT, it must have occurred in the mitochodrias or the singlet oxygen must have entered the nucleus by diffusion. It is, however, more likely that the photosensitizer was bound to the cellular membranes and the cell death was caused by damaging the membrane by the singlet oxygen. Our finding could support this hypothesis by showing a high fluorescence intensity in the perinuclear area, where the nuclear membrane and the endoplasmatic reticulum are located.

The survival curves described here provide a basis for determining lethal and sublethal doses of photosensitizer plus red-light combinations both for more detailed preclinical studies and for clinical purposes, e.g., estimation of sublethal PDT doses for the study of cells that survive treatment. The form of the survival curves on the semilog-plots, figures 3 and 4, display a nonlinear decrease in survival. The 1 μg/ml m-THPC curves decreased to a plateau even after modest laser light fluence-doses. A doubling of the laser light intensity caused little enhanced cytotoxicity. This may result from photobleaching of the photosensitizer. The dye has been shown to be destroyed by the laser light [7]. The effect was most pronounced in the V-79 fibroblasts. It has been suggested that this is the reason why PDT displays a modest selectivity for malignant cells [10]. Other effects of PDT on biological end-points such as cell transformation, damage repair, the influence of the cell cycle on the uptake of the photosensitizer, and others that may effect cell survival levels have not yet been investigated.

A comparison of the sensitivity of the two cell lines to PDT revealed enhanced cytotoxicity of V-79 cells to higher concentrations of photosensitizer and higher laser-light intensities compared to MCF-7 cells. This suggests an unfavourable discrimination by PDT between malignant breast carcinoma cells and nonmalignant lung fibroblasts. Although the V-79 cells are nonmalignant, they are immortal, which accounts for their proliferative behaviour being similar to that of malignant cells [19]. In agreement with other authors [20,21], V-79 cells were found to be a suitable biological model system for the in vitro studies. Both cell lines are appropriate for PDT studies. They are easy to handle and have short cycle times. It is important that the various centres working with PDT use the same lines in the same manner if reliable interlaboratory comparisons or reproducibility of data are to be attempted. Such efforts have not yet been routinely performed.

ACKNOWLEDGMENTS

We thank Mrs. M. Widmer for excellent photographic work. A part of the results were presented as a poster at the Congress of the Swiss Society of Gynecology and Obstetrics, Davos, June 21–24, 1995. We thank Dr. M. Grahn and Dr. Ch. Stewart for editorial advice, and Prof. T. Gasser, Division of Biostatistics, University of Zürich, for statistical support.

REFERENCES

1. Policard MA. Etudes sur les aspects offerts par des tumeurs experimentales examinées à la lumière de Wood. Soc Biol 1924; 91:1423–1424.
2. Dougherty TJ. Photosensitizers: Therapy and detection of malignant tumors. Photochem Photobiol 1987; 45:879–889.
3. Moan J, Berg K. Yearly review: Photochemotherapy of cancer: Experimental research. Photochem Photobiol 1992; 55:931–948.
4. Berg K, Moan J. Photodynamic effects of Photofrin II on cell division in human NHIK 3025 cells. Int J Radiat Biol 1988; 5:781–797.
5. Bonnett R, White RD, Winfield UJ, Berenbaum MC. Hydroporphyrins of the mesotetra(hydroxyphenyl)porphyrin series as tumor photosensitizers. Biochem J 1989; 261:277–280.
6. Bonnett R, Berenbaum M. Porphyrins as photosensitizers. Ciba Found Symp 1989; 146:40–53.
7. Ris H-B, Altermatt HJ, Nachbur B, Stewart JCM, Wang Q, Lim CK, Bonnett R, Althaus U. Effect of drug-light intervall on photodynamic therapy with meta-tetrahydroxyphenylchlorin in malignant mesothelioma. Int J Cancer 1993; 53:141–146.
8. Wang Q, Ris H-B, Altermatt HJ, Reynolds B, Stewart JCM, Bonnett R, Lim CK. Determination of 5,10,15,20-Tetra-(m-hydroxyphenyl)chlorin in Tissues by High Performance Liquid Chromatography. Biomed Chromatogr 1993; 7:155–157.
9. Puck TT, Marcus PI. A rapid method for viable cell titration and clone production with HeLa cells in tissue culture: The use of X-irradiated cells to supply conditioning factors. Biochemistry 1955; 41:432–437.
10. Ris H-B, Altermatt HJ, Stewart CM, Schaffner T, Wang Q, Lim CK. Photodynamic therapy with m-tetrahydroxyphenylchlorin in vivo: optimization of the therapeutic index. Int J Cancer 1993; 55:245–249.
11. Steichen JD, Weiss MJ, Elmaleh DR, Martuza RL. Enhanced in vitro uptake and retention of 3H-tetraphe-

nylphophonium by nervous system tumor cells. J Neurosurg 1991; 74:116–122.
12. Ris H-B, Altermatt HJ, Inderbitzi R, Hess R, Nachbur B, Stewart JCM, Wang Q, Lim CK, Bonnett R, Berenbaum MC, Althaus U. Photodynamic therapy with chlorins for diffuse malignant mesothelioma: initial clinical results. Br J Cancer 1991; 64:1116–1120.
13. Yu W, Naim JO, Lanzafame RJ. The effect of laser irradiation on the release of bFGF from 3T3 fibroblasts. Photochem Photobiol 1994; 59:167–170.
14. Henderson BW, Dougherty TJ. How does photodynamic therapy work? Photochem Photobiol 1992; 55(1):145–157.
15. Van Breugel HHFI, Bär PR. He-Ne laser irradiation affects proliferation of cultured rat Schwann cells in a dose-dependent manner. J Neurocytol 1993; 22:185–190.
16. Dobler-Girdziunaite DD, Burkard W, Haller U, Larsson B, Walt H. Kombinierte Anwendung der Photodynamischen Therapie mit ionisierenden Strahlen bei Mammakarzinom-Zellen in vitro. Strahlenther. Onkol 1995; 171:622–629.
17. Kvam E, Stokke T, Moan J, Steen HB. Plateau distribution of DNA fragments lengths produced by extended light exposure of extranuclear photosensitizers in human cells. Nucleic Acids Res 1992; 20:6687–6693.
18. Dobler-Girdziunaite DD, Major A, Haller U, Walt H. Korrelation zwischen der Fluoreszenzintensität und der Wirkung der photodynamischen Therapie (PDT) bei gynäkologischen Tumorzellen in vitro. Arch Gynecol Obstet 1995; 256 (Suppl):S229.
19. Andreoni A, Cubeddu R, De Silvestri S, Laporta P, Ambesi-Impiombato FS, Esposito M, Mastrocinque M, Tramontano D. Effects of laser irradiation on hematoporphyrin-treated normal and transformed thyroid cells in culture. Cancer Res 1983; 43:2076–2080.
20. Brasseur N, Nguyen T-L, Langlois R, Ouellet R, Marengo S, Houde D, Van Lier JE. Synthesis and photodynamic activities of silicon 2,3-naphthalocyanine derivatives. J Med Chem 1994; 37:415–420.
21. Margaron P, Langlois R, Van Lier JE. Photodynamic properties of naphthosulfobenzoporphyrazines, novel asymmetric, amphiphilic phthalocyanine derivatives. J Photochem Photobiol B 1992; 14:187–199.
22. Wijnaendts van Resandt RW, Marsman HJB, Kaplan R, Davoust J, Stelzer EHK, Stricker R. Optical fluorescence microscopy in three dimensions: microtomoscopy. J Microsc 1985; 138:29–34.
23. Baak JPA, Thunnissen FBJM, Oudejans CBM, Schipper NW. Potential clinical uses of laser scan microscopy. Appl Optics 1987; 26:3413–16.